

# Cell culture and reagents

MG Melanie Girard

Updated date: Dec 15, 2020

 An abbreviated version of this protocol was published in Science Immunology in Nov 2020

Type I interferons drive the maturation of human DC3s with a distinct costimulatory profile characterized by high GITRL

DOI: 10.1126/sciimmunol.abe0347

## Detailed protocol

### Culture of cryopreserved human PBMCs with IFN- $\beta$

1. Thaw a 1 ml vial of  $10^7$  cryopreserved human PBMCs in a 37°C water bath
2. Transfer the cells into a conical tube with 14 ml complete media
3. Centrifuge cells at 1400 rpm 4°C for 7 minutes and discard supernatant
4. Resuspend cell pellet in 10 ml complete media
5. Count cells on a hemacytometer
6. Centrifuge cells at 1400 rpm 4°C for 7 minutes and discard supernatant
7. Resuspend cells at a concentration of  $2 \times 10^6$  cells/ml
8. Transfer 500  $\mu$ l ( $10^6$ ) cells/well in a 24-well plate
9. Prepare a 2X solution of IFN- $\beta$  in complete media (500U/mL)
10. Add 500  $\mu$ l of 2X cytokine solution or 500  $\mu$ l of complete media on top of cells (final concentration is 250 U/mL)
11. Culture cells for 18-24h at 37°C 5% CO<sub>2</sub>

### Complete media

- RPMI 1640
- 10% fetal bovine serum
- 1 mM sodium pyruvate
- 100 U/mL penicillin
- 0.1 mg/mL streptomycin
- 1% non-essential amino acids (Gibco)
- 0.1% mL 2-mercaptoethanol

### Cytokines

- Recombinant human IFN- $\beta$  (Peprotech)

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Girard, M. (2020). Cell culture and reagents. Bio-protocol Preprint. [bio-protocol.org/prep701](https://bio-protocol.org/prep701).
2. Girard, M., Law, J. C., Edilova, M. I. and Watts, T. H. (2020). Type I interferons drive the maturation of human DC3s with a distinct costimulatory profile characterized by high GITRL . Science Immunology 5(53). DOI: [10.1126/sciimmunol.abe0347](https://doi.org/10.1126/sciimmunol.abe0347)

**Copyright:** Content may be subjected to copyright.